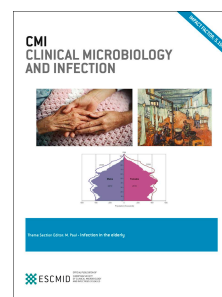


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**Analysis of clinical and environmental *Candida parapsilosis* isolates by  
microsatellite genotyping – a tool for hospital infections surveillance**

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**Running Title:** Microsatellite genotyping of clinical and environmental *Candida  
parapsilosis* isolates

**Abstract**

*Candida parapsilosis* emerged as an important opportunistic pathogen, causing candidemia worldwide. Nosocomial outbreaks triggered by this species have been frequently described, particularly in cancer patients. For a better understanding of its epidemiology, several typing methods are used and microsatellite analysis has been reported as highly discriminant. The main objective of this work was to study *C. parapsilosis* isolates by application of microsatellite genotyping to distinguish epidemiologically related strains, compare clinical and environmental isolates and determine possible routes of dispersion of the isolates in the hospital setting. A total of 129 *C. parapsilosis* isolates from different origins, including hospital environment and hands of health care workers, were genotyped using four microsatellite markers. The isolates were recovered from different health institutions. Analysis of *C. parapsilosis* isolates from hospital environment showed great genotypic diversity, however the same or very similar genotypes were also found. The same multilocus genotype was shared by isolates recovered from the hand of a healthcare worker, from the hospital environment, and from patients of the same health care institution, suggesting that these could be possible routes of transmission and that infections due to *C. parapsilosis* may be mainly related with exogenous transmission to the patient. Examination of sequential isolates from the same patients showed that colonizing and bloodstream isolates had the same multilocus genotype in the majority of cases. We demonstrate that this typing method is able to distinguish clonal clusters from genetically unrelated genotypes and can be a valuable tool to support epidemiological investigations in the hospital setting.

**Keywords:**

*Candida parapsilosis*, microsatellite genotyping, nosocomial infections, health care workers, hospital air and surfaces.

**Introduction**

Infections caused by *Candida* species have been rising in the last decades mainly due to the multiple medical resources used currently, such as chemotherapy, antibiotics, catheters and parenteral nutrition [1-5]. Although *C. albicans* continues to be the most common cause of bloodstream infections, longitudinal studies have detected a shift towards non-*albicans* *Candida* infections, specifically *C. glabrata*, *C. tropicalis* and *C. parapsilosis* [1, 6, 7].

*Candida parapsilosis* is associated with approximately 25% of *Candida* infections in European hospitals and it is now the second most commonly isolated *Candida* species from bloodcultures in Europe, Canada and Latin America, and in some European hospitals even outranks *C. albicans* [5, 7]. Invasive disease caused by *C. albicans* and *C. tropicalis* is normally preceded by prior colonization while, in contrast, invasive disease produced by *C. parapsilosis* can occur without prior colonization and is frequently transmitted horizontally via contaminated external sources such as infusates [8], the hands of health care workers [9, 10], prosthetic devices [11], catheters [12-14], parenteral hyperalimentation [15, 16]. It is possible that inanimate environmental surfaces and the colonized patients themselves constitute a reservoir from which other patients may acquire the fungus and in some cases develop candidemia [17, 18]. *Candida parapsilosis* is a particular problem as it

tends to grow as biofilms on implanted medical devices, conferring almost total resistance to antifungal drugs, and this ability to grow as a biofilm seems to be directly related with the capacity to cause clinically significant disease [19].

The laboratory identification and typing of pathogens is frequently used by epidemiologists for providing evidence for the biological and genetic relatedness of these organisms as an aid in the epidemiological investigation of nosocomial infections. The rationale for such subspecies or strain delineation is that repeated isolation of the same strain from one or more patients suggests that the organism may have originated from a single clone and thus is more likely to represent infection in the case of a single patient or transmission from patient to patient from a common source or by a common mechanism [20]. The genetic relationship between clinical isolates, route of acquisition, nosocomial transmission, or the emergence of antifungal resistant strains can be studied by using DNA-based typing methods and the need to discriminate among *C. parapsilosis* strains has been reported in several studies for a better understanding of the epidemiology of this species [10, 21, 22]. However, most of the genotyping methods are not discriminant enough to distinguish closely related isolates and to establish routes of transmission.

A microsatellite-based typing method to genotype *C. parapsilosis* sensu stricto isolates, using four loci composed by tandemly repetitive stretches of three nucleotides, has been recently described, achieving a discriminatory power of 99.9% [23]. Our aim was the study of *C. parapsilosis* isolates with different origins by application of these microsatellite markers in order to distinguish epidemiologically related isolates, identify prevalent strains in the hospital setting, compare between environmental and clinical isolates and determine possible routes of acquisition of a

strain in the hospital environment or identify possible reservoirs. We demonstrate that this typing method is able to distinguish clonal clusters from genetically unrelated genotypes and can be a valuable tool to support epidemiological investigations.

## Materials and methods

### *Yeast strains and DNA extraction*

A total of 129 *C. parapsilosis* strains with different origins were analysed in this study (Table 1). Several isolates were collected from the same patient in different time periods or in the same period but from different biological products. In these cases, clinical presentation of the patients and dates of collection are presented in Tables 4 and 5. The environmental *C. parapsilosis* isolates analysed were actively collected from the Haematology ward of an oncological hospital during four sampling periods (January, April, September and February), in the same time period and at the same time that isolates from the patients were collected as well. Isolates from the air were collected using an air sampler (Millipore) with a velocity air rate of 140 L/min. The sampled air (500 m<sup>3</sup>) was directly impacted onto 2% malt extract agar (MEA) with chloramphenicol (0.05 g/L). Samples from hard surfaces were collected by swabbing them with a cotton swab pre-moistened with sterile saline solution, according to the International Standard ISO 18593 [24]. The sample swabs were then streaked onto MEA. Collection of the Isolates from the hands of health care workers (14 nurses, 4 physicians, 6 medical auxiliaries or hospital technicians, 4 members of the administrative staff) was previously approved by the Ethical

committee of the selected hospital). Those isolates were collected using contact plates at the palm of the hand and swabs in nails and inter-digital spaces of the hands. *Candida parapsilosis* isolates were identified using the methodology previously described [23, 25]. Stock cultures were maintained on Sabouraud glucose agar medium at 4°C. Prior to DNA isolation, yeast cells were grown for 24 hours on Sabouraud glucose agar medium at 37°C. DNA extraction was performed by using the High Pure PCR Template kit (Roche Diagnostics Corp., Indianapolis, Ind.), according to the manufacturer's instructions.

#### *PCR amplification conditions fragment size determination.*

Four *C. parapsilosis* specific microsatellite markers were used, designated by CP1, CP4, CP6 and B and PCR amplification was performed following the methodology developed and described previously [23].

Following PCR, denaturated samples were run in an ABI 310 Genetic Analyser (AB Applied Biosystems) and the PCR products size was determined by using the GeneScan 3.7 Analysis software and alleles were designated by their sizes in base pairs (bp) [23].

#### *Statistical analysis.*

Allelic and genotypic frequencies were determined by using ARLEQUIN ver.2.000 software. Genetic distance between *C. parapsilosis* isolates was calculated by using shared allele distance (DAS) in the Populations 1.2.30 software program. The clustering of the isolates was performed with NTSys software, using UPGMA method.

## Results

### *Analysis of Candida parapsilosis isolates from the hospital environment and healthcare workers*

Air from the Haematology unit of an oncological hospital, as well as from several hard surfaces, such as doors knobs, bedside tables, water taps and medical trolleys of the same unit were sampled and screened for *C. parapsilosis*. The hands of health care workers of that same ward were also analysed and several *C. parapsilosis* isolates were collected. Great genotype diversity was observed amongst hospital environmental isolates (18 different multilocus genotypes), however some of them were found to share the same multilocus genotype (M, O, P and F, Q, R) (Table 2).

Isolates M, O and P were all collected in the same day, one of them from a medical trolley and the other two from doors knobs. The places from where these isolates were collected suggest a possible strain transmission due to manual handling (Table 3). Interestingly, none of the patients of the studied hospital unit presented infections due to *C. parapsilosis* with a similar multilocus genotype. Isolate F was collected from a health care worker hand in January/07 and presented the same multilocus genotype as an isolate recovered from a water tap and another one collected from a medical trolley in April/07 (Table 3).

In opposition to what was observed in the previous case, two patients presented infections caused by isolates with the same multilocus genotype. The first one was hospitalized in the same hospital ward in May/07 and the strain was isolated from a bloodculture. The other clinical isolate presenting the same genotype was collected in August/05 from the pus of a patient hospitalized in the Gastroenterology Unit. A



dendrogram showing the degree of genetic similarity based on microsatellite genotyping, among the clinical and environmental *Candida parapsilosis* isolates obtained in that hospital is presented in Fig. 1.

#### *Analysis of isolates from the same patients and possible occurrence of an outbreak*

Sequential *C parapsilosis* isolates obtained from the same patients in different time periods, as well as multiple isolates from the same patient recovered from different biological products were analysed in six hospitalized patients. Table 4 shows the clinical data associated with each patient and the microsatellite multilocus genotypes obtained in each case.

In four of the studied patients, the sequential isolates presented similar genotypes: from patient #1 three isolates were recovered from bloodcultures separated by about one month from each other and patient #3 presented one isolate from the catheter and another one from a bloodculture. The same multilocus genotype was observed in these isolates indicating that they were the same or very similar strains. . Patient #4 presented a positive bloodculture preceded by a positive urine culture two days before, and the infecting strains displayed the same genotype. From patient #6, isolates were recovered from different places and dates but all the strains shared the same multilocus genotype.

The scenario was different regarding the remaining cases whose isolates collected from the same patient presented different genotypes: in patient #2 it was observed that the multilocus genotypes of the two isolates recovered were not the same. However, the difference was only observed in one of the locus (CP6), indicating that

the strains causing these two infections were very closely related. From patient #5, the isolate collected from the skin and the one recovered from the blood were different strains since they presented distinct genotypes. Therefore, most probably these two strains were acquired from different sources and the strain colonizing the skin was not the cause of the bloodstream infection.

It was observed that three *C. parapsilosis* isolates collected from blood and urine of three different patients hospitalized in the Paediatrics Unit at the same time, within a period of 20 days, shared the same multilocus genotype indicating that they were the same or very closely related strains (Table 5). The presence of the same strain in three different patients at the same period of time suggests the possible occurrence of an outbreak in that hospital unit. Interestingly, no other *C. parapsilosis* strain was isolated during that period in the same unit and, as no further sampling was performed, it was not possible to trace the origin of the outbreak.

#### *Global analysis and most common genotypes*

The 129 *C. parapsilosis* isolates analysed in this study included 45 from bloodcultures and catheter tips, 31 from skin and nails, 29 from other body sources and 24 from air and surfaces of the hospital environment. A great genotypic diversity was found among the isolates, corresponding to 108 different multilocus genotypes. The most frequent genotypes found in the different biological products are presented in Table 6.

The most common multilocus genotypes in isolates from bloodcultures are 222/243 300/300 282/336 127/127 and 240/252 300/300 285/285 147/149, shared by three (6.6%) isolates each. The first corresponds to the strains isolated from the

described possible outbreak in the Paediatric Unit (Table 5). Interestingly, the second was only observed in five strains from the French hospital suggesting the possibility of a local or resident strain.

In the case of the isolates from skin and nails, the most common genotype was 222/243 354/354 282/336 129/129 and shared by seven (22.5%) isolates. One of these isolates was collected from a HCW hand of the Haematology Unit studied and the other six were recovered from six different non-hospitalized patients and in different dates, suggesting that this clone could be particularly adapted to skin and nails. Regarding other clinical products, the multilocus genotype 222/243 354/354 282/282 129/129 is the only one that is shared by three (10.3%) isolates. From the environmental strains, two multilocus genotypes are shared by three (12.5%) isolates each: multilocus genotypes 240/243 342/342 285/285 103/103 and 240/240 342/342 285/285 103/103. In this case, three of the isolates were collected from the air of the same health institution and the other three correspond to the isolates M, O and P (Table 3).

Globally, the multilocus genotype 222/243 354/354 282/336 129/129 was the most frequently found, shared by 11 isolates. These isolates were collected from the hospital environment (two of them), from the hands of a health care worker (one isolate), from hospitalized patients (pus and blood) and from non-hospitalized patients mainly originated from skin and nails (7). These isolates were collected in two different and independent Portuguese health institutions and were not observed in isolates from other geographical origins.

## Discussion

In order to determine the possibility of infection or transmission it is necessary that the isolates of *Candida parapsilosis* can be exactly discriminated at the strain level since the results of genotyping methods with low discriminatory potential may lead to misleading ideas concerning the surveillance of candidiasis [26]. Thus, distinguishing among strains of *C. parapsilosis* is crucial to understand the epidemiology of this pathogen [27]. The amount of genetic variation between isolates gives a measure of their relatedness and molecular typing is performed to determine whether different isolates give the same or different results for one or more tests. Epidemiologically related isolates share the same DNA profile or fingerprint, whereas sporadic or epidemiologically unrelated isolates have distinctly different patterns [28]. According to several studies that have been performed to distinguish *C. parapsilosis* strains, ITS group I /RFLP subtype VII-1/ *C. parapsilosis sensu stricto* isolates have high genome homogeneity [21, 22, 27, 29, 30], which was considered a difficulty to perform epidemiological studies with this organism. Microsatellite markers have been applied in the study of clinical yeast species and have shown a high discriminatory power in discriminating *C. albicans* strains [18, 22, 31-36]. In 2007, Lasker *et al.* [22] described a set of seven dinucleotidic microsatellites markers, able to discriminate *C. parapsilosis sensu stricto* strains, with a discriminatory power of 0.97. Brillowska-Dabrowska and colleagues [37] used a combined methodological approach with pyrosequencing, multilocus sequence typing, random amplified polymorphism and microsatellite genotyping to study a *C. parapsilosis* nosocomial outbreak in a haematology ward and they concluded that the last one appears to be the highest resolution method.

The polymorphic microsatellite markers described by Sabino *et al.* [23] showed a higher discriminatory power than other methodologies and were applied in the study of several possible outbreaks [38-41]. In the present work they were used to distinguish among epidemiologically related isolates and to study several *C. parapsilosis* populations.

When applied to clinical isolates and when sequential isolates from patients were genotyped, in four of six cases, the series of isolates displayed the same genotype. The maintenance of multilocus genotypes was also observed by other authors in infections due to *Candida albicans* [33, 34, 42]. We detected at a given time, in the same patient, strains presenting identical multilocus genotype isolated from multiple anatomical sites, and also a colonizing strain and a bloodstream isolate, from the same patient, sharing the same multilocus genotype. These observations are in accordance to what is generally agreed stating that candidaemia usually arises as an endogenous infection following prior colonization of the gastrointestinal tract, skin, or vagina [43, 44]. Colonizing strains, however, are not always the source of infection. In contrast to what happens with *C. albicans*, infections by *C. parapsilosis* may occur without prior colonization of the patients [22]. That fact was observed in patient #5, from whom *C. parapsilosis* skin isolate did not share the same multilocus genotype than the blood isolate, suggesting that the bloodstream infection might have been of exogenous source. In fact, it has been reported that clustering of *Candida* strains in time and space may result from cross-infection from an exogenous source, usually transmitted by contaminated healthcare workers [8].

Molecular typing methods have illustrated the link between hand carriage of *C. parapsilosis* and the horizontal transmission and outbreak of infections of *C.*

*parapsilosis* in hospital environments by showing the genetic similarities among isolates from health care workers and clinical isolates [9, 10]. High degree of genetic similarity was found among isolates collected from the hospital environment and hands of healthcare workers. This could be observed particularly in the case of isolates F, Q and R, where one of them was collected from the hand of a healthcare worker, two with similar genotype from the hospital ward environment, and other two from patients of the same health care institution. All these isolates shared the same multilocus genotype which was also the most common genotype found. It is a genotype well established in this hospital and the hypothesis that its route of transmission is through the hands of healthcare workers and the contaminated medical trolleys and doors knobs cannot be ruled out. Moreover, this genotype was found in 2005 and 2007 and according with Shimdit *et al.* [45], a long-term success of a given strain is enhanced if it is broadly adaptable. The other environmental isolates collected in the same hospital ward displayed multilocus genotypes that were not found in the patients isolates, indicating that in these cases the infections might not have been hospital acquired.

The presence of a prevalent genotype could be due to the lack of a sexual cycle in *C. parapsilosis* and to the expansion of some clones [22, 46], and may also suggest a development of a global dominance of a single *C. parapsilosis* genotype.

Although microsatellite multiplex genotyping is highly discriminatory, the possible convergence of ancestral alleles to the same length by different mutational events, an effect known as homoplasy, can be a limiting factor for strain identification. Nevertheless, as several loci are considered, the high microsatellite variability often largely compensates for their eventual homoplastic evolution. Thus, the application of

microsatellite loci in studies such as molecular epidemiology and population studies is highly recommended since the accurate discrimination of genetically divergent groups within pathogenic species is critical to the appropriate development and use of treatment strategies [47].

In conclusion, the four *C. parapsilosis* microsatellite markers used in this study are sufficiently polymorphic to allow a high discriminatory power thus permitting their application in epidemiological studies for recurrent infections and nosocomial outbreaks. The results obtained in the present study have proven to be very valuable in studying the genetic relatedness among *C. parapsilosis* isolates, which can be easily differentiated with high discriminatory power, allowing the detection of outbreaks.

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### References

[1] Safdar A, van Rhee F, Henslee-Downey JP, Singhal S, Mehta J. *Candida glabrata* and *Candida krusei* fungemia after high-risk allogeneic marrow transplantation: no adverse effect of low-dose fluconazole prophylaxis on incidence and outcome. Bone Marrow Transplant 2001;28:873–878.

[2] Pemán J, Cantón E, Gobernado M, The Spanish ECMM Working Group on Candidemia. Epidemiology and antifungal susceptibility of *Candida* species isolated from blood: results of a 2-year multicentre study in Spain. Eur J Clin Microbiol Infect Dis 2004;23:1267-1275.

[3] Bedini, A, Venturelli C, Mussini C, *et al.* Epidemiology of candidaemia and antifungal susceptibility patterns in an Italian tertiary-care hospital. Clin Microbiol Infect 2006;12:75-80.

[4] Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis and treatment. Med Mycol 2007;45:321-346.

[5] Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev 2007;20:33-163.

[6] Capoor MR, Nair D, Deb M, Verma PK, Srivastava L, Aggarwal P. Emergence of non-*albicans* *Candida* species and antifungal resistance in a tertiary care hospital. Jpn J Infect Dis 2005;58:344-348.

[7] Almirante B, Rodriguez D, Park BJ, *et al.* Epidemiology and predictors of mortality in cases of *Candida* bloodstream infection: results from population-based surveillance, Barcelona, Spain, from 2002 to 2003. J Clin Microbiol 2005;43:1829-1835.



[8] San-Miguel LG, Pla J, Cobo J, Navarro F, Sánchez-Sousa A, Alvarez ME, *et al.* Morphotypic and genotypic characterization of sequential *Candida parapsilosis* isolates from an outbreak in a pediatric intensive care unit. *Diagn Microbiol Infect Dis* 2004;49:189-196.

[9] Lupetti A, Tavanti A, Davini P, Ghelardi E, Corsini V, Merusi I, *et al.* Horizontal transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. *J Clin Microbiol* 2002;40:2363-2369.

[10] van Asbeck, EC, Huang YC, Markham AN, Clemons KV, Stevens DA. *Candida parapsilosis* fungemia in neonates: genotyping results suggest healthcare workers hands as source, and review of published studies. *Mycopathologia* 2007;164:287–293.

[11] Kan C-D, Luo C-Y, Lin P-Y, Yang Y-J. Native-valve endocarditis due to *Candida parapsilosis*. *Interact Cardiovasc Thorac Surg* 2002;1:66–68.

[12] Raad II, Bodey GP. Infectious complications of indwelling vascular catheters. *Clin Infect Dis* 1992;15:197-210.

[13] Levin AS, Costa SF, Mussi NS, Levin AS1, Costa SF, Mussi NS, Basso M, Sinto SI, *et al.* *Candida parapsilosis* fungemia associated with implantable and semi-implantable central venous catheters and the hands of healthcare workers. *Diagn Microbiol Infect Dis* 1998;30:243-249.

[14] Deshpande K. *Candida parapsilosis* fungaemia treated unsuccessfully with amphotericin b and fluconazole but eliminated with caspofungin: a case report. Crit Care Res 2003;5:20-23.

[15] Weems JJ Jr, Chamberland ME, Ward J, Willy M, Padhye AA., and Solomon SL. *Candida parapsilosis* fungemia associated with parenteral nutrition and contaminated blood pressure transducers. J Clin Microbiol 1987;25:1029-1032.

[16] Trofa D, Gácsér A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. Clin Microbiol Rev 2008;21:606–625.

[17] Hota B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? Clin Infect Dis 2004;39:1182–1189.

[18] Stephan F, Bah MS, Desterke C, Rézaiguia-Delclaux S, Foulet F, Philippe Duvaldestin P, *et al.* Molecular diversity and routes of colonization of *Candida albicans* in a surgical intensive care unit, studied using microsatellite markers. Clin Infect Dis 2002; 35:1477-1483.

[19] Laffey SF, Butler G. Phenotype switching affects biofilm formation by *Candida parapsilosis*. Microbiology 2005;151:1073-1081.

[20] Pfaller MA. Molecular approaches to diagnosing and managing infectious diseases: practicality and costs. Emerg Infect Dis 2001;7:312-318.

[21] Tavanti A, Davidson AD, Gow NA, Maiden MC, Odds FC. *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. J Clin Microbiol 2005;43:284–292.

[22] Lasker BA, Butler G, Lott TJ. Molecular genotyping of *Candida parapsilosis* Group I clinical isolates by analysis of polymorphic microsatellite markers. J Clin Microbiol 2006;44:750-759.

[23] Sabino R, Rosado L, Sampaio P, Stevens DA, Clemons KV, Pais C. New polymorphic microsatellite markers able to distinguish among *Candida parapsilosis* sensu stricto isolates. J Clin Microbiol 2010;48:1677-1682.

[24] ISO 18593:2004: Microbiology of food and animal feeding stuffs -- Horizontal methods for sampling techniques from surfaces using contact plates and swabs. International Organization for Standardization (ISO). Geneva, International Organization for Standardization ISO, and Brussels, Euro-pean Committee for Standardization CEN, September 2003.

[25] Correia A, Sampaio P, Almeida J, Pais C. Study of molecular epidemiology of candidiasis in Portugal by PCR fingerprinting of *Candida* clinical isolates. J Clin Microbiol 2004;42:5899-5903.

[26] Dalle F, Lafon I, L'Ollivier C, Ferrant E, Sicard P, Labruère C, et al. A prospective analysis of the genotypic diversity and dynamics of the *Candida albicans* colonizing flora in neutropenic patients with de novo acute leukemia. *Haematologia* 2008; 93:581-587.

[27] van Asbeck EC, Clemons KV, Stevens DA. *Candida parapsilosis*: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. *Crit Rev Microbiol* 2009;35:283-309.

[28] Pfaller MA. Epidemiological typing methods for mycoses. *Clin Infect Dis* 1992; 14:S4-10.

[29] Shin JH, Shin DH, Song JW, Kee SJ, Suh SP, Ryang DW. Electrophoretic karyotype analysis of sequential *Candida parapsilosis* isolates from patients with persistent or recurrent fungemia. *J Clin Microbiol* 2001;39:1258-1263.

[30] van Asbeck EC, Clemons KV, Markham AN, Stevens DA. Correlation of restriction fragment length polymorphism genotyping with internal transcribed spacer sequence, randomly amplified polymorphic DNA and multilocus sequence groupings for *Candida parapsilosis*. *Mycoses* 2009;52:493-498.

[31] Dalle F, Franco N, Lopez J, Vagner O, Caillot D, Chavanet P, Cuisenier B, et al. Comparative genotyping of *Candida albicans* bloodstream and non bloodstream isolates at a polymorphic microsatellite locus. *J Clin Microbiol* 2000;38:4554-4559.

[32] Dalle F, Dumont L, Franco N, Mesmacque D, Caillot D, Bonnin P, et al. Genotyping of *Candida albicans* oral strains from healthy individuals by polymorphic microsatellite locus analysis. J Clin Microbiol 2003;41:2203–2205.

[33] Eloy O, Marque S, Botterel F, Stephan F, Costa J-M, Lasserre V, et al. Uniform distribution of three *Candida albicans* microsatellite markers in two French ICU populations supports a lack of nosocomial cross-contamination. BMC Infect Dis 2006;6:162-169.

[34] Sampaio P, Gusmão L, Correia A, Alves C, Rodrigues AG, Pina-Vaz C, et al. New microsatellite multiplex PCR for *Candida albicans* strain typing reveals microevolutionary changes. J Clin Microbiol 2005;43:3869-76.

[35] Foulet F, Nicolas N, Eloy O, Botterel F, Gantier JC, Costa JM, et al. Microsatellite marker analysis as a typing system for *Candida glabrata*. J Clin Microbiol 2005; 43:4574-4579.

[36] Garcia-Hermoso D, Maccallum DM, Lott TJ, Sampaio P, Buitrago Serna MJ, Grenouillet F, et al. A multicenter collaborative study for the standardization of *Candida albicans* genotyping using a polymorphic microsatellite marker. J Clin Microbiol 2010;8:2578-2581

[37] Brillowska-Dabrowska A, Pannanusorn ST, Lönnbro N, et al. A nosocomial outbreak of *Candida parapsilosis* in southern Sweden verified by genotyping. Scand J Infect Dis 2009;41:135-142.

[38] Vaz C, Sampaio P, Clemons KV, Huang YC, Stevens DA, Pais C. Microsatellite multilocus genotyping clarifies the relationship of *Candida parapsilosis* strains involved in a neonatal intensive care unit outbreak. Diagn Microbiol Infect Dis 2011; 71:159-62.

[39] Romeo O, Delfino D, Cascio A, Lo Passo C, Amorini M, Romeo D, et al. Microsatellite-based genotyping of *Candida parapsilosis sensu stricto* isolates reveals dominance and persistence of a particular epidemiological clone among neonatal intensive care unit patients. Infect Genet Evol 2013;13:105–108.

[40] Diab-Elschahawi M, Forstner C, Hagen F, Meis JF, Lassnig AM, Presterl E, et al. Microsatellite genotyping clarified conspicuous accumulation of *Candida parapsilosis* at a cardiothoracic surgery intensive care unit. J Clin Microbiol 2012; 50:3422-3426.

[41] Delfino D, Scordino F, Pernice I, Lo Passo C, Galbo R, David A, et al. Potential association of specific *Candida parapsilosis* genotypes, bloodstream infections and colonization of health workers' hands. Clin Microbiol Infect 2014;20:O946-O951.

[42] Sampaio P, Gusmão L, Alves C, Pina-Vaz C, Amorim A, Pais C. Highly polymorphic microsatellite for identification of *Candida albicans* strains. J Clin Microbiol 2003;41:552-557.

[43] Traoré O, Springthorpe V, Sattar S. A quantitative study of the survival of two species of *Candida* on porous and non-porous environmental surfaces and hands. J Appl Microbiol 2002;92:549-555.

[44] Miranda LN, van der Heijden IM, Costa SF, Sousa AP, Sienna RA, Gobara S, et al. *Candida* colonisation as a source for candidaemia. J Hosp Infect 2009;72:9-16.

[45] Schmid J, Herd S, Hunter PR, et al. Evidence for a general-purpose genotype in *Candida albicans*, highly prevalent in multiple geographical regions, patient types and types of infection. Microbiology 1999;145:2405–2413.

[46] van Asbeck EC, Clemons KV, Markham AN, Stevens DA and The *Candida parapsilosis* Global Epidemiology Group. Molecular epidemiology of the global and temporal diversity of *Candida parapsilosis*. Scand J Infect Dis 2008;40:827-834.

[47] Sampaio P, Correia A, Gusmão L, Amorim A, Pais C. Sequence analysis reveals complex mutational processes for allele length variation at two polymorphic microsatellite loci in *Candida albicans*. In: Méndez-Vilas A, ed. Communicating Current Research and Educational Topics and Trends in Applied Microbiology. Badajoz: Formatex, 2007:926-935.

**Table 1.** Origin of the *Candida parapsilosis* isolates

| Origin of the isolates          | Portuguese institutions | French institutions | Total |
|---------------------------------|-------------------------|---------------------|-------|
| Bloodcultures and catheter tips | 21                      | 24                  | 45    |
| Skin and nails                  | 28                      | 3                   | 31    |
| Other biological products       | 21                      | 8                   | 29    |
| Hospital environment            | 22                      | 2                   | 24    |
| Total                           | 92                      | 37                  | 129   |



Table 2. Microsatellite multilocus analysis of *Candida parapsilosis* isolates obtained from the environment of a hospital ward and their health care workers

| Isolate<br>identification | Source                              | Date     | Multilocus Genotypes |           |           |           |
|---------------------------|-------------------------------------|----------|----------------------|-----------|-----------|-----------|
|                           |                                     |          | CP1                  | CP4       | CP6       | B         |
| A                         | Nursery 3 water tap                 | Jan-2007 | 234 / 240            | 318 / 318 | 267 / 267 | 145 / 147 |
| B                         | Nursery 3 Bedside table 4           | Jan-2007 | 222 / 246            | 354 / 357 | 336 / 336 | 129 / 129 |
| C                         | Nursery 2 water tap                 | Jan-2007 | 240 / 243            | 327 / 342 | 285 / 285 | 143 / 145 |
| D                         | HCW hands                           | Jan-2007 | 240 / 246            | 375 / 375 | 333 / 333 | 127 / 127 |
| E                         | HCW hands                           | Jan-2007 | 240 / 243            | 360 / 360 | 273 / 321 | 129 / 129 |
| F                         | HCW hands                           | Jan-2007 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| G                         | Nursery 2 Bedside table             | Jan-2007 | 240 / 243            | 294 / 294 | 273 / 291 | 127 / 133 |
| H                         | Nursery 3 Bedside table1            | Jan-2007 | 240 / 243            | 294 / 294 | 273 / 294 | 127 / 133 |
| I                         | Patient W.C. - shower               | Jan-2007 | 240 / 243            | 327 / 339 | 285 / 285 | 143 / 145 |
| J                         | Nursery 2 door knob                 | Jan-2007 | 237 / 240            | 309 / 309 | 261 / 279 | 145 / 147 |
| K                         | Patient's W.C.                      | Jan-2007 | 237 / 240            | 399 / 399 | 285 / 306 | 129 / 129 |
| L                         | Nursery 1 Bedside table             | Jan-2007 | 237 / 240            | 342 / 342 | 285 / 285 | 105 / 105 |
| M                         | Patient individual room's door knob | Apr-2007 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| O                         | Patient W.C door knob               | Apr-2007 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| P                         | Nursery Medical trolley             | Apr-2007 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| Q                         | Water tap (treatment room)          | Apr-2007 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| R                         | Nursery medical trolley             | Apr-2007 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| S                         | HCW hands                           | Sep-2007 | 237 / 246            | 297 / 297 | 276 / 276 | 127 / 133 |
| T                         | Air from individual room no.5       | Feb-2008 | 237 / 243            | 297 / 297 | 276 / 294 | 127 / 133 |
| U                         | Water tap (inside) treatment room   | Feb-2008 | 243 / 243            | 309 / 309 | 264 / 279 | 145 / 145 |
| V                         | Shower Patients' WC                 | Feb-2008 | 222 / 243            | 354 / 354 | 282 / 282 | 129 / 129 |
| X                         | Air from nursery 24                 | Feb-2008 | 237 / 243            | 297 / 297 | 276 / 276 | 133 / 133 |

**Table 3.** Common multilocus microsatellite genotypes of *Candida parapsilosis* isolates obtained from the environment of a hospital ward and from their health care workers.

| Isolate Identification  | Source                            | Hospital ward    | Date     | Multilocus Genotypes |           |           |           |
|-------------------------|-----------------------------------|------------------|----------|----------------------|-----------|-----------|-----------|
|                         |                                   |                  |          | CP1                  | CP4       | CP6       | B         |
| M                       | Patient individual room door knob | Haematology      | 11/04/07 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| O                       | Patients W.C. door knob           | Haematology      | 11/04/07 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| P                       | Nursery medical trolley           | Haematology      | 11/04/07 | 240 / 240            | 342 / 342 | 285 / 285 | 103 / 103 |
| F                       | HCW hands                         | Haematology      | 20/01/07 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| Q                       | Water tap                         | Haematology      | 11/04/07 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| R                       | Nursery medical trolley           | Haematology      | 11/04/07 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| Patient isolate H972697 | Bloodculture                      | Haematology      | 04/05/07 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| Patient isolate G730127 | Pus                               | Gastroenterology | 05/08/05 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |

**Table 4.** Microsatellite multilocus analysis of several sequential isolates obtained from the same patients.

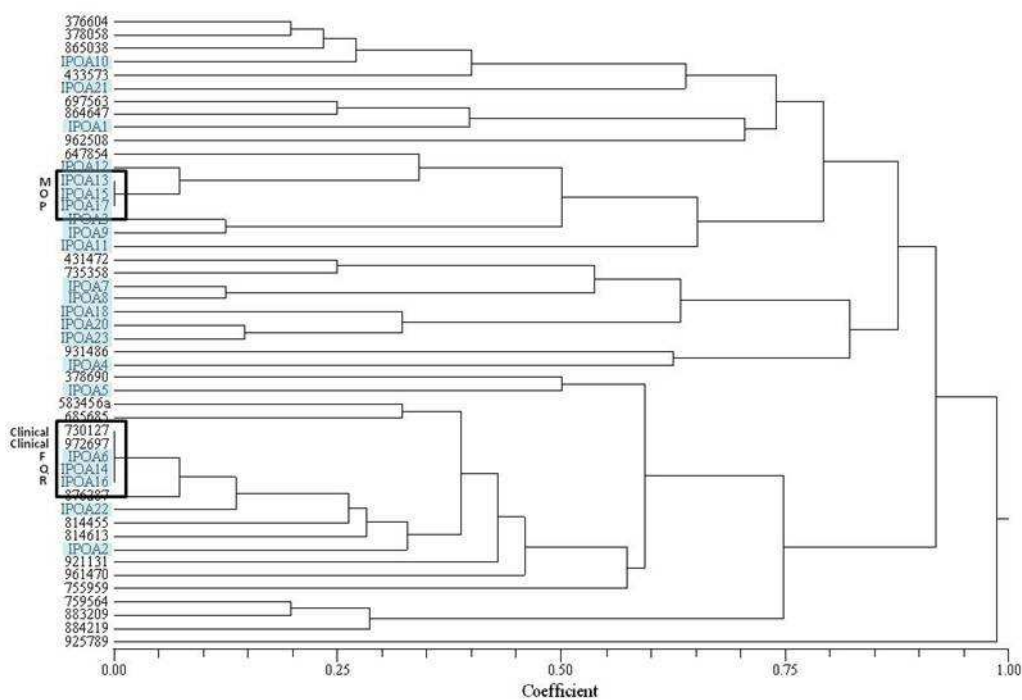
| Patient # | Origin              | Patient's data      | Clinical data                | Biological Product       | Date     | Multilocus Genotypes |           |           |           |
|-----------|---------------------|---------------------|------------------------------|--------------------------|----------|----------------------|-----------|-----------|-----------|
|           |                     |                     |                              |                          |          | CP1                  | CP4       | CP6       | B         |
| 1         | Portuguese hospital | Male, 17 years old  | Acute lymphoblastic leukemia | Bloodculture             | 24/11/03 | 240 / 240            | 354 / 354 | 282 / 282 | 129 / 129 |
|           |                     |                     |                              | Bloodculture             | 30/12/03 | 240 / 240            | 354 / 354 | 282 / 282 | 129 / 129 |
|           |                     |                     |                              | Bloodculture             | 12/01/04 | 240 / 240            | 354 / 354 | 282 / 282 | 129 / 129 |
| 2         | Portuguese hospital | Female 55 years old | Thyroid carcinoma            | Bloodculture             | 04/04/06 | 222 / 243            | 354 / 354 | 279 / 279 | 129 / 129 |
|           |                     |                     |                              | Bloodculture             | 04/05/07 | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 |
| 3         | French Hospital     | Female 56 years old | Liver carcinoma              | Bloodculture             | 27/06/03 | 240 / 252            | 300 / 300 | 285 / 285 | 147 / 149 |
|           |                     |                     |                              | Catheter                 | 30/06/03 | 240 / 252            | 300 / 300 | 285 / 285 | 147 / 149 |
| 4         | French Hospital     | Male 46 years old   | Peritonitis, laparotomy      | Urine                    | 21/03/06 | 243 / 243            | 264 / 297 | 267 / 267 | 131 / 131 |
|           |                     |                     |                              | Bloodculture             | 23/03/06 | 243 / 243            | 264 / 297 | 267 / 267 | 131 / 131 |
| 5         | French Hospital     | Male 73 years old   | Lung carcinoma               | Skin                     | 23/09/05 | 240 / 252            | 240 / 252 | 177 / 195 | 147 / 149 |
|           |                     |                     |                              | Bloodculture             | 24/09/05 | 240 / 252            | 300 / 300 | 279 / 285 | 147 / 149 |
| 6         | French Hospital     | Male 75 years old   | Aorto-femoral bypass         | Abdominal drain effluent | 20/12/06 | 243 / 258            | 318 / 357 | 297 / 297 | 141 / 141 |
|           |                     |                     |                              | Tracheal aspirate        | 27/12/06 | 243 / 258            | 318 / 357 | 297 / 297 | 141 / 141 |
|           |                     |                     |                              | Oropharyngeal swab       | 09/01/07 | 243 / 258            | 318 / 357 | 297 / 297 | 141 / 141 |

**Table 5.** Clinical data and multilocus genotype of the *Candida parapsilosis* isolates regarding a possible outbreak in a hospital unit.

| Patient # | Sex    | Age | Clinical data   | Hospital unit | Outcome  | Biological Product | Collection Date | Genotypes |           |           |           |
|-----------|--------|-----|-----------------|---------------|----------|--------------------|-----------------|-----------|-----------|-----------|-----------|
|           |        |     |                 |               |          |                    |                 | CP1       | CP4       | CP6       | B         |
| 1         | Female | 12  | Ewing sarcoma   | Pediatrics    | Died     | Urine              | 29/08/03        | 222 / 243 | 354 / 354 | 282 / 336 | 127 / 127 |
| 2         | Male   | 3   | Lymphocytosis   | Pediatrics    | Survived | Bloodculture       | 08/09/03        | 222 / 243 | 354 / 354 | 282 / 336 | 127 / 127 |
| 3         | Male   | 13  | Liver Carcinoma | Pediatrics    | Survived | Bloodculture       | 17/09/03        | 222 / 243 | 354 / 354 | 282 / 336 | 127 / 127 |

**Table 6.** Multilocus genotypes most frequently found in the different biological products.

| Origin of the isolates                 | Multilocus genotypes |           |           |           | No. Isolates (%) |
|--|----------------------|-----------|-----------|-----------|------------------|
|  | CP1                  | CP4       | CP6       | B         |                  |
| Bloodcultures and catheter tips (n=45) | 222 / 243            | 354 / 354 | 282 / 336 | 127 / 127 | 3 (6.6)          |
|  | 240 / 252            | 300 / 300 | 285 / 285 | 147 / 149 | 3 (6.6)          |
| Skin and nails (n=31)                  | 222 / 243            | 354 / 354 | 282 / 336 | 129 / 129 | 7 (22.5)         |
| Other biological products (n=29)       | 222 / 243            | 354 / 354 | 282 / 282 | 129 / 129 | 3 (10.3)         |
|  | 240 / 240            | 342 / 342 | 285 / 285 | 105 / 105 | 3 (12.5)         |
| Hospital environment (n=24)            | 240 / 243            | 342 / 342 | 285 / 285 | 103 / 103 | 3 (12.5)         |



**Figure 1.** Dendrogram showing the clustering of clinical and environmental *Candida parapsilosis* isolates from the same hospital, based on microsatellite multilocus genotyping. Genetic distances were calculated by using Populations 1.2.30 software program and clustering performed by using UPGMA method ( $r=0.91357$ ). The isolates highlighted are environmental strains.